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Redox-Dependent Differential Optimization of Contractile Work in Cardiac Muscle from Diabetic Rat under Hyperglycemia

Niraj M. Bhatt, Miguel A. Aon, Xiaoxu Shen, Brian O'Rourke, Wei Dong Gao, Sonia Cortassa.
Division of Cardiology, Johns Hopkins Uiversity School of Medicine, Baltimore, MD, USA.

Type 2 diabetes mellitus (T2DM) is characterized by obesity, hyperglycemia and insulin resistance; these traits are recapitulated by the Zucker Diabetic Fatty (ZDF) rat model. Although fatty acid oxidation is known to be augmented in the heart, it is unclear how substrate fuel selection and redox behavior affect optimization of cardiac work output in T2DM. We studied the mechanical, and energetics/redox behavior of heart trabeculae from ZDF rats and their lean controls perfused with either normal (euglycemia, EG, 5mM) or high glucose (HG, 30mM), in the absence or presence of Palmitate (Palm). Contractile performance, measured as developed force and area under the muscle tension curve (contractile work), increased with frequency, as did oxygen consumption rate (VO_2). The ratio of contractile work over VO_2 was defined as an index of the energy yield for contractile work (Ycw). Unlike in lean animals, ZDF heart trabeculae showed a significant increase in Ycw under HG in the presence of Palm. On the contrary, ZDF trabeculae perfused under EG in the presence of Palm exhibited lower Ycw than in lean animals, in agreement with published data. The improved function with Palm under HG was associated with improved redox status in ZDF trabeculae, but not in lean controls. Indeed, MCB fluorescence, an indicator of cellular GSH levels, and the probe of oxidative stress, CM-DCF, revealed more oxidizing redox conditions under HG that could be reverted by the addition of Palm in ZDF trabeculae. In contrast with the behavior under EG, Palm elicited an increased reliance of contractility on glycolysis in conjunction with oxidative phosphorylation under HG in ZDF but not in lean trabeculae. As a result, Palm induces a redox-dependent higher contractile work yield from respiration in muscles from diabetic rat hearts.

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Cardiomyocytes from Creatine-Deficient Mice Lacking L-Arginine: Glycine Amidinotransferase (AGAT) Show No Changes in Mitochondrial Organization and Cellular Compartmentation

Svetlana Kotlyarova[1], Merle Mandel[1], Niina Sokolova[1], Dunja Aksentijevic[2], Craig A. Lygate[2], Stefan Neubauer[2], Marko Vendelin[1], Rikke Birkedal[1].
[1]Institute of Cybernetics at TUT, Tallinn, Estonia, [2]University of Oxford, Oxford, United Kingdom.

In cardiac muscle, the creatine kinase (CK) system temporally buffers ADP and ATP concentrations near sites of ATP production and consumption. It has also been suggested to be an important spatial buffer facilitating the transport of ADP and ATP. This was corroborated by a study of CK knockout mice. However, a recent study on cardiomyocytes from creatine-deicient guanidinoacetate methyltransferase (GAMT) knockout mice showed no effect on mitochondrial organization and compartmentation. It has been suggested that in GAMT knockout mice, accumulated guanidinoacetate can be used as a substrate instead of creatine. Therefore, we studied the same parameters in L-arginine: glycine amidinotransferase (AGAT) mice, which are also creatine-deficient, but do not accumulate guanidinoacetate. Three-dimensional mitochondrial organization at whole cell level was assessed by confocal microscopy. Kinetic measurements on permeabilized cardiomyocytes included the affinity of oxidative phosphorylation to exogenous ADP and ATP, competition between mitochondria and pyruvate kinase for ADP produced by ATPases, ADP-kinetics of endogenous pyruvate kinase and ATP-kinetics of ATPases. Our breeding records show that AGAT-/- mice are less viable than their wild-type congeners. Most died at four to five months of age. In cardiomyocytes, visual inspection of mitochondrial organization suggests no essential difference between AGAT knockout and wildtype mice. Also, there is no difference in the kinetics of respiration and ATPases. Thus, our data so far suggest no differences in cardiomyocyte mitochondrial organization and cellular compartmentation between AGAT knockout and wildtype mice. We conclude that the reduced viability of AGAT mice is most likely not due to cardiac failure. The lack of compensatory changes in mitochondrial organization and cellular compartmentation in AGAT as well as GAMT mice raise questions regarding the importance of the CK system as a spatial buffer.

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Distribution of Intracellular ADP Diffusion Restriction in Trout Cardiomyocytes

Niina Sokolova, Mervi Sepp, Marko Vendelin, Rikke Birkedal.
Tallinn University of Technology, Tallinn, Estonia.

Cardiomyocytes are compartmentalized by intracellular diffusion restrictions. In our previous study we confirmed that isolated permeabilized cardiomyocytes from rainbow trout (Oncorhynchus mykiss) also display intracellular diffusion restriction (Sokolova et al., BMC Cell Biology, 10:90, 2009). This was surprising, because trout cardiomyocytes are much thinner than those of mammals, lack t-tubules, have a lower sarcoplasmic reticulum density and only a single layer of myofilaments surrounding a central core of mitochondria. However, the extent of diffusion restriction is much smaller than in mammalian cardiomyocytes. The aim of the present study was to find the possible distribution of diffusion restrictions in permeabilized trout cardiomyocytes. We measured mitochondrial respiration stimulated by ADP and ATP, and evaluated the rate of inhibition of ATP stimulated respiration by an ADP trapping system, consisting of phosphoenolpyruvate (PEP) activating endogenous and exogenous pyruvate kinase (PK), which competes with mitochondria for ADP. We found a high activity of hexokinase, which stimulates mitochondrial respiration when activated by glucose. To study its role in more detail, all experiments were performed in the absence and presence of 2 mM glucose. Additionally, we performed ADP titrations on cells alone, in the presence of glucose, and in the presence of creatine kinase and creatine. We found that activation of hexokinase by glucose, but not activation of creatine kinase by creatine, lowered the apparent ADP-affinity in permeabilized trout cardiomyocytes. This is in contrast to the situation in permeabilized mammalian cardiomyocytes, where activation of mitochondrial creatine kinase has a profound effect on the apparent ADP-affinity. This suggests that trout cardiomyocytes have a different ADP-feedback system than mammalian cardiomyocytes. The experimental results will be fitted with a mathematical model (Sepp et al., Biophysical Journal, 98:12, 2010) showing more specifically the distribution and extent of compartmentalization of trout cardiomyocytes.

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An Extra-Mitochondrial Domain Rich in Carbonic Anhydrase Activity Improves Myocardial Energetics

Marie A. Schroeder[1], Mohammad Ali[1], Alzbeta Hulikova[1], Claudiu T. Supuran[2], Kieran Clarke[1], Richard D. Vaughan-Jones[1], Damian J. Tyler[1], Pawel Swietach[1].
[1]University of Oxford, Oxford, United Kingdom, [2]University of Florence, Florence, Italy.

CO_2 is produced in vast quantities by cardiac mitochondria and an efficient means of its venting is required to support metabolism. Carbonic anhydrases (CAs), expressed at various sites in ventricular myocytes, may affect mitochondrial CO_2-clearance by catalyzing CO_2 hydration (to H^+ and HCO_3^-) and changing trans-membrane [CO_2]-gradients for diffusion. using fluorescent dyes to measure pH-changes arising from the intracellular hydration of extracellularly-supplied CO_2, overall CA activity in the cytoplasm of isolated ventricular myocytes was found to be modest (2.7-fold above spontaneous kinetics). Experiments on isolated ventricular mitochondria demonstrated negligible intra-mitochondrial CA activity. Cardiac CA activity was also investigated by ^{13}C magnetic resonance spectroscopy (MRS) from the rate of production of $H^{13}CO_3^-$ from $^{13}CO_2$, released by mitochondrial metabolism of hyperpolarized [1-^{13}C]pyruvate. CA activity measured upon [1-^{13}C] pyruvate infusion was four-fold higher than the cytoplasm-averaged value. However, the apparent CA activity decreased after $^{13}CO_2$ was allowed to dissipate away from its mitochondrial source. A fluorescent CA-ligand co-localized with the mitochondrial marker TMRE, indicating that mitochondria are near a CA-rich domain. Based on immunoreactivity, this domain may comprise CAXIV and, to a lesser extent CAII, which remained closely associated with mitochondria after a purification procedure. Extra-mitochondrial CA activity raised matrix pH (flow-cytometry of isolated mitochondria) and improved cardiac energetics indexed by increased phosphocreatine-to-ATP ratio and decreased [ADP] (^{31}P-MRS of intact hearts). These data provide evidence for a functional domain of high CA activity around mitochondria to support CO_2-venting. Aberrant CA activity or distribution may reduce the heart's energetic efficiency.

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Translocation of Glycolytic ATP into Mitochondria of Cancer Cells does not Utilize the Adenine Nucleotide Transporter

Eduardo N. Maldonado[1], Joe Vuicich[2], David N. DeHart[2], Heather S. Rodebaugh[2], John J. Lemasters[1].
[1]Center for Cell Death, Injury & Regeneration, Department of Drug Discovery & Biomedical Sciences; Hollings Cancer Center; Medical University of South Carolina, Charleston, SC, USA, [2]Center for Cell Death, Injury & Regeneration, Medical University of South Carolina, Charleston, SC, USA.

BACKGROUND: Cancer cells utilize aerobic glycolysis rather than oxidative phosphorylation to generate most cellular ATP (Warburg phenomenon). In non-transformed differentiated cells, the adenine nucleotide translocator (ANT) catalyzes exchange of ATP for ADP across the mitochondrial inner membrane. Bongkrekic acid (BA) and carboxyatractyloside (CATR) specifically inhibit ANT. Here, our AIM was to assess whether mitochondrial ATP translocation in cancer cells depends on ANT.
METHODS: Mitochondrial membrane potential ($\Delta\Psi$) was assessed by confocal microscopy of tetramethylrhodamine methylester (TMRM) fluorescence. Respiration by HepG2 and A549 cells was determined with a Seahorse XF24 Analyzer. RESULTS: In rat hepatocytes, respiratory inhibition by myxothiazol (MYX) slightly decreased $\Delta\Psi$, but subsequent oligomycin (OL), BA or CATR collapsed $\Delta\Psi$, indicating that mitochondrial hydrolysis of glycolytic (cytosolic) ATP sustains $\Delta\Psi$. In HepG2 and A549 cells, MYX also slightly decreased $\Delta\Psi$, and subsequent OL collapsed $\Delta\Psi$. By contrast to hepatocytes, BA and CATR added after MYX did not collapse $\Delta\Psi$, whereas 2-deoxyglucose (2-DG), a glycolytic inhibitor, added after MYX, MYX+BA and MYX+CATR did collapse $\Delta\Psi$. OL but not BA or CATR alone decreased respiration in both cell lines, whereas BA and CATR inhibited hepatocyte respiration. ANT2 is the predominant ANT isoform expressed in cancer cells, and 2-DG after MYX in ANT2 knockdown cells depolarized mitochondria.
CONCLUSION: In cancer cells ANT is not the principal ATP transporter responsible for mitochondrial uptake of glycolytic ATP from the cytosol. Moreover, ANT2 deficiency does not alter uptake of glycolytic ATP into mitochondria. Warburg metabolism, therefore, appears to utilize an alternative pathway for entry of ATP into mitochondria.

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Experimental and Simulation Analysis of NADH-Enzymes Binding in a Crowded Environment
Travis Fransen[1], Monica Soto Velasquez[2], Robb Welty[1], Dhanushka Wickramasinghe[1], **Ahmed Heikal**[1].
[1]Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN, USA, [2]Department of Chemistry and Biochemistry, The College of St. Scholastica, Duluth, MN, USA.
Reduced nicotinamide adenine dinucleotide (NADH) is a key coenzyme used in many metabolic pathways such as glycolysis and oxidative phosphorylation in living cells. The intracellular fractions of free and enzyme-bound NADH have been shown to be sensitive to mitochondrial activities of brain tissues and cancer cells. In this contribution, we investigate the effects of molecular crowding on the reaction kinetics of NADH and lactate dehydrogenase (LDH) using two-photon, time-resolved fluorescence anisotropy. Synthetic polymers (Ficoll-70) and proteins (bovine serum albumin, BSA, and ovalbumin) were used as biomimetic crowding agents as compared with homogeneous buffer. In addition, computer simulations coupled with reaction kinetics were used to guide our experimental design and data interpretation. The observed associate anisotropy of NADH-LDH mixture depends on both the type and concentration of crowding agents. Complementary measurements on intracellular NADH in C3H10T1/2 cells, under resting condition, were also carried out. These non-invasive, time-resolved associated anisotropy results elucidate the role of molecular crowding on NADH-LDH interactions in both biomimetic environment and living cells. Our findings will ultimately help establishing intracellular NADH as a natural biomarker for a myriad of biochemical reactions as well as diagnostic tool for mitochondrial anomalies (i.e., health).

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Metabolic Profiling of Multicell Tumor Spheroids by NADH Fluorescence and Spatially-Resolved Oximetry
Michael G. Nichols, Lyandysha V. Zholudeva, Marcus J. Lehnerz, Danielle E. Desa, Christian T. Meyer.
Creighton University, Omaha, NE, USA.
While fluorescence intensity-based metabolic imaging techniques have provided a useful means of monitoring cellular energetics, recent work has demonstrated that fluorescence lifetime imaging (FLIM) provides additional details into the subcellular trafficking of energy intermediates within the cell. Specifically, FLIM can measure the reorganization of NADH within distinct subcellular pools with change in the metabolic state induced by inhibitors, uncouplers and substrate availability. Here, we compare NADH-intensity and FLIM measurements of metabolism of cells grown as either monolayer culture or 300-500 μm diameter multicell tumor spheroids in media under different growth conditions. These measures are correlated with cellular respiration monitored using an oxygen-sensitive electrodes to test the hypothesis that NADH FLIM-based metabolic imaging more accurately measures the cellular metabolic state of three-dimensional living tissue than intensity-based measurements.
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Application of FRET Biosensors in Energy Metabolism
Martin Pelosse[1], Hiromi Imamura[2], Imre Berger[3], Uwe Schlattner[1].
[1]Joseph Fourier University and Inserm, Grenoble, France, [2]Kyoto University, Kyoto, Japan, [3]EMBL, Grenoble, France.
Genetically encoded optical biosensors become a tool of choice for quantitative studies on distribution and concentration changes of ions and metabolites in living cells. In systems biology, they are expected to provide multi-scale analysis in space and time for an advanced understanding of both normal and diseased physiological states. Here we develop and apply fluorescent biosensors based on fluorescence resonance energy transfer (FRET) that are able to monitor directly or indirectly the cellular energy state. Such genetically encoded FRET sensors allow quantitative analysis of changes in adenylate pools or activation of signaling pathways triggered by such changes. This should yield new insight into the spatiotemporal organization of cellular energy metabolism.

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A Minimal Model of Ubiquinol:Cytochrome C Reductase Capable of Simulating Superoxide Production
Jason Bazil[1], Kalyan C. Vinnakota[1], Wu Fan[2], Daniel A. Beard[1].
[1]Medical College of Wisconsin, Milwaukee, WI, USA, [2]CFD Research Corporation, Huntsville, AL, USA.
Ubiquinol:Cytochrome c reductase (complex III) is an enzyme in the respiratory chain of mitochondria that serves as a critical link between ubiquinol-generating enzymes and cytochrome c oxidase, the terminal enzyme in the chain. During pathological conditions, it is implicated in the generation of reactive oxygen species (ROS) and thus induction of oxidative stress. Herein, a biophysically-detailed, thermodynamically-consistent model of complex III in mammalian mitochondria is presented. The model incorporates all major redox centers near the Qo- and Qi-site of the enzyme; includes the pH-dependence of all redox mediated reactions; and ROS production at the Qo-site. The model consists of distinct states characterized by the electron distribution in the enzyme. within each state, sub-states that correspond to various electron localizations exist in rapid equilibrium with each other. The steady-state equation derived from the state model was parameterized using five independent data sets. Model analysis suggests that the pH-dependence on turnover is primarily due to the pKa's of the heme bL and Rieske ISP. Also, previously proposed kinetic scheme at the Qi-site where quinone only binds to the reduced enzyme and quinol only binds to the oxidized enzyme is shown to be thermodynamically infeasible. Moreover, the model is able to reproduce the bi-stability phenomenon whereby different rates of ROS production are maintained when the enzyme is differentially reduced while the overall flux through the enzyme is the same. Integrating this model into existing mitochondrial respiration models would produce more realistic predictions and help uncover the intricate relationship between ROS production and mitochondrial bioenergetics.

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Mechanistic Electron Transport Chain Model Explains ROS Production in Different Respiratory Modes
Laura D. Gauthier, Sonia Cortassa, Joseph L. Greenstein, Raimond L. Winslow.
Johns Hopkins University, Baltimore, MD, USA.
Reactive oxygen species (ROS) have been implicated in disorders ranging from neurodegenerative diseases to diabetes to heart disease. In cardiac myocytes mitochondria represent the predominant source of ROS, specifically complexes I and III. The model presented here endeavors to explore and elucidate the modulation of electron transport chain ROS production under state 3 versus state 4 respiration and the role of succinate as a substrate. A mechanistic complex III model was developed, driven by redox potential differences between adjacent redox centers. This model shows that ROS production increases exponentially with membrane potential when in state 4. Because the mechanism of ROS production from complex I remains unknown, a more general thermodynamic model was used to describe the influence of $NADH/NAD^+$ and ubiquinone/ubiquinol redox potentials on complex I-derived ROS release. This release occurs in the presence of NADH and succinate, leading to a highly reduced ubiquinone pool, displaying the highest ROS production flux in state 4. Overall, total ROS production is moderate